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Carcinogenic Risk from Nitrite, Nitrate and N-Nitrosamines in Food

An important aspect of the public health problem of cancer is the identification of carcinogens in the environment, and the assessment of whether they represent a significant health hazard. As a contribution to the solution of this problem research with the N-nitroso compounds is seeking to answer two questions: (1) Is man exposed to amounts of N-nitroso compounds sufficient by themselves, or together with other carcinogens, to cause cancer? (2) Can the study of nitrosamine-induced cancer in animals help us to identify other chemical carcinogens in the environment and assess their significance, even if the N-nitroso compounds themselves play no part in the induction of cancer in man?

Nitrite can react readily with amines and amides to form N-nitroso compounds, most of which are highly carcinogenic (Druckrey *et al.* 1967). Nitrate does not react in this way, and nitrate is relatively nontoxic, but nitrate has to be considered in this context because it can be reduced to nitrite, commonly by bacterial action. This reduction occurs during bacterial spoilage of nitrate-containing food, and the bacteria both in the mouth of the adult, and in the upper gastrointestinal tract of the human infant, are also able to reduce nitrate to nitrite (for review *see* Swann 1975). Nitrite is used as a preservative in cured meat and cheese (Binkerd & Kolari 1975) and previously it was thought that this food additive was the major source of nitrite in man's diet. However, it has recently been realized that most of man's intake of nitrite comes from reduction of the nitrate in the saliva (White 1975).

Nitrosamines have been found in human food, and the nitrosation reaction, which proceeds most rapidly in weak acid (Mirvish 1970, 1971), can also occur when dietary amines and nitrite are mixed in the stomach (Sander 1971). Because they are potent carcinogens effective in most animal species, and in most organs, efforts have been made to discover whether man is exposed to appreciable amounts of carcinogenic nitroso compounds. This endeavour has had to face considerable difficulties, both practical and theoretical.

The practical difficulty is one of analysis. It is not yet possible successfully to analyse complex mixtures, such as food, for nonvolatile N-nitroso compounds, and even the analysis for volatile N-nitrosamines requires careful extraction and expensive equipment such as the gas chromatograph and mass spectrometer (Foreman & Goodhead 1975). However, new and more sensitive techniques of analysis are now becoming available and a more complete assessment of the amount of nitrosamines in the environment may soon be available (Fine & Rounbehler 1975).

The theoretical problem has already been mentioned. It is now well established that the administration of amines and nitrite by mouth results in the formation of carcinogenic N-nitroso compounds in the stomach. If it is true that for man the major source of nitrite is the saliva, then it is possible that the major part of man's exposure to nitroso compounds comes from compounds synthesized in his own stomach by the reaction between dietary amines and the nitrite in his saliva. Since the extent of the possible contribution of nitrosamine formation in the stomach is not known, the figures showing only the nitrosamine content of food may considerably underestimate man's exposure to these substances.

Analysis has shown that nitrosamines can be found in several human foods. Dimethylnitrosamine, diethylnitrosamine, and nitrosopyrrolidine, have been found in a few samples of fish, fish products and cheese, and in a greater number of samples of cured meat and bacon. These foods (fish, cheese and cured meat) were selected for examination because it was thought that they were those most likely to contain nitrosamines, but even so more than half the samples did not contain detectable amounts. In almost all the other samples the amount of nitrosamine was less than 100 μg/kg, indeed most contained less than 5 μg/kg (Egan & Hubbard 1975, Scanlan 1975). Nitrosamines have also been found in tobacco and tobacco smoke (Hecht *et al.* 1975) and apparently also in the atmosphere of some American cities, but again the amounts found were small.

It should be remembered that the method of analysis measures only relatively stable and volatile nitrosamines; but none the less, the quantities found are very much lower than the lowest dietary concentration of dimethylnitrosamine known to be carcinogenic to the rat (about 2 μg/kg diet). To assess the significance of these traces of nitrosamine we need to know the shape of the dose response curve at these very low doses; we need to know whether amounts too small to induce a measureable incidence of cancer in animals are absorbed through the wall of the intestinal tract to reach the remote organs in an active form; and above all we need to know whether these doses are cumulative in their effect and to what extent there is cooperation in carcinogenic action between one nitrosamine and another, and between nitrosamines and other carcinogens.

The dose response curve for dimethylnitrosamine-induced liver cancer is now being carefully studied by the British Industrial Biological

Research Association. An experiment of this kind is costly and difficult to carry out, yet it gives information on the effect of only one carcinogen in only one animal species and still leaves the problem of extrapolating the results to man. It is clear that the more complex problems of assessing the cumulation of doses and the cooperation between different carcinogens will not be solved by animal experimentation unless those experiments are illuminated by an understanding of the mechanism of chemical carcinogenesis. Fortunately there is now rapid progress in this understanding.

There is now evidence that the nitroso compounds owe their carcinogenic activity to their ability to be converted *in vivo* into alkylating agents which react with cellular components. Even though the exact role of the alkylation reactions in the carcinogenic process is not yet known, this incomplete knowledge has allowed an approach to one of the problems outlined above. If dimethylnitrosamine is present in the diet, alkylation of components of organs such as the liver will occur only if the carcinogen passes through the intestinal wall without being inactivated. By measuring alkylation in the liver Diaz Gomez *et al.* (1977) has shown that if a rat is given an oral dose even of less than 1 μg, dimethylnitrosamine passes from the stomach to the liver without appreciable loss.

The studies on the alkylation of cellular components have recently become focused on the alkylation of the O^6-position of guanine in DNA. The O^6-position is involved in the normal Watson-Crick base-pairing, and alkylation of this position is mutagenic. There are two lines of argument which lead one to suspect that this particular alkylation is most important in carcinogenesis. The preliminary studies which have recently been carried out suggest that the carcinogenic activity of alkylating agents is broadly related to the ability of each agent to alkylate this position (Lawley 1974), and that the sensitivity of each organ to the carcinogenic action of nitroso compounds reflects the ability of each organ to remove the carcinogen-produced O^6-alkylguanine from its DNA (Goth & Rajewsky 1974, Kleihues & Margison 1974, Margison & Kleihues 1975, Nicoll *et al.* 1975).

It was previously shown that the effect of doses of carcinogen is cumulative and irreversible (Druckrey 1967), but a recent study of the cumulation of the carcinogenic effect of divided doses suggests that there can be substantial repair of the carcinogenic damage induced by dimethylnitrosamine (Swann *et al.* 1976). This result could be reconciled with those of Druckrey and would be compatible with the experiments indicating the importance of the alkylation of the O^6-position of DNA in carcinogenesis. It is possible that the repair being demonstrated reflects the enzymic removal of this altered base from the DNA. However if this altered base is present at DNA synthesis a permanent, essentially irreversible, mutation will be induced in the DNA of the progeny cells. This irreversible lesion might cumulate with subsequent mutations and account for Druckrey's result.

Thus if it is finally proven that this reaction is responsible for the carcinogenic activity of these compounds, it will provide a conceptual basis for an understanding of the cumulation of carcinogenic doses and the cooperation between carcinogens. If it is so proved, and if other chemical carcinogens work in a way similar to the nitroso compounds, then preliminary searches for environmental carcinogens may be carried out on the assumption that such carcinogens produce analogous changes in DNA. It is true that the carcinogenic activity of a chemical is influenced by other properties, such as its pharmacokinetics, but the success of the Ames test (Ames *et al.* 1973) indicates the potential of a measurement of DNA-damaging properties as a screening test of environmental chemicals for possible carcinogenic activity.

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Carcinogenesis of Vinyl Chloride

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(see *Proceedings of the Royal Society of Medicine*, 1976, **69**, 281)

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